UNCLASSIFIED

DTIC FILE COPY (2)

SECURITY CLASSIFICATION OF THIS PAGE

REPORT DOCUMENTATION PAGE

AD-A188 678

1b. RESTRICTIVE MARKINGS

3. DISTRIBUTION / AVAILABILITY OF REPORT
APPROVED FOR PUBLIC RELEASE, DISTRIBUTION UNLIMITED

4. PERFORMING ORGANIZATION REPORT NUMBER(S)

5. MONITORING ORGANIZATION REPORT NUMBER(S)
AFOSR-TR- 87-1411

6a. NAME OF PERFORMING ORGANIZATION
MARIO NEGRI INSTITUTE FOR PHARMACOLOGICAL RESEARCH

6b. OFFICE SYMBOL (If applicable)

7a. NAME OF MONITORING ORGANIZATION
AFOSR/NL

6c. ADDRESS (City, State, and ZIP Code)
Via Eritrea, 62
20157 MILANO, ITALY

7b. ADDRESS (City, State, and ZIP Code)
Building 410
Bolling AFB, D.C. 20332-6448

8a. NAME OF FUNDING / SPONSORING ORGANIZATION
AFOSR

8b. OFFICE SYMBOL (If applicable)

9. PROCUREMENT INSTRUMENT IDENTIFICATION NUMBER
AFOSR-85-0196

8c. ADDRESS (City, State, and ZIP Code)
Building 410
Bolling AFB DC 20332-6448

10 SOURCE OF FUNDING NUMBERS

PROGRAM ELEMENT NO.	PROJECT NO.	TASK NO	WORK UNIT ACCESSION NO.
61102F	2312	A5	

11 TITLE (Include Security Classification)
2,3,7,8-TETRACHLORODIBENZO-P-DIOXIN INDUCED IMMUNODEPRESSION : ITS POSSIBLE ALTERATION BY IN VIVO ADMINISTRATION OF SPECIFIC HEPATIC ENZYME INDUCERS

12 PERSONAL AUTHOR(S) Annunciata VECCHI

13a. TYPE OF REPORT	13b. TIME COVERED	14. DATE OF REPORT (Year, Month, Day)	15 PAGE COUNT
FINAL	FROM 5/1/1985 TO 4/30/87	1987, June 27	

16 SUPPLEMENTARY NOTATION

17 COSATI CODES			18 SUBJECT TERMS (Continue on reverse if necessary and identify by block number)
FIELD	GROUP	SUB-GROUP	HEPATIC INDUCERS, IMMUNODUPPRESSION, ANTIBODY PRODUCTION, CELLULAR REACTIVITIES

19 ABSTRACT (Continue on reverse if necessary and identify by block number)

The possible alterations of TCDD toxicity induced by treatments with cytochrome P-450 inducers such as phenobarbital, 3-Methylcholanthrene, beta-naphtoflavone and 2,3,7,8-tetrachlorodibenzofuran (TCDF) have been investigated in mice. TCDD toxicity has been evaluated in terms of immunosuppression and hepatic enzyme induction. Drugs able to interfere with the immune response, that is 3-Methylcholanthrene, beta-naphtoflavone and TCDF, usually increased the level of inhibition of immune responses when given at the same time as TCDD. Treatment with 3-Methylcholanthrene before or after TCDD always resulted in additive immunosuppressive effect. Similar results were observed in enzymatic studies. In TCDD-TCDF combination when TCDD was given 2 day before or after TCDF, the effect observed in animals treated with both toxins usually was not statistically different from the effects (inhibition of immune parameters or enzymatic induction) caused by the most active compound used

20 DISTRIBUTION / AVAILABILITY OF ABSTRACT
☒ UNCLASSIFIED/UNLIMITED ☒ SAME AS RPT ☐ DTIC USERS

21 ABSTRACT SECURITY CLASSIFICATION
UNCLASSIFIED

22a NAME OF RESPONSIBLE INDIVIDUAL
Lorris G. Cockerham, Lt. Col

22b TELEPHONE (Include Area Code)
767-5021

22c OFFICE SYMBOL
NL

DD FORM 1473, 84 MAR 83 APR edition may be used until exhausted. All other editions are obsolete.

SECURITY CLASSIFICATION OF THIS PAGE

19. ABSTRACT - (continued)

in that condition. Time-course experiments were consistent with the conclusion that inducer usually did not modify TCDD toxicity. If inducers had toxic effects by themself, the effect of combined treatments was the sum of the effect of each drug.

ISTITUTO DI RICERCHE FARMACOLOGICHE «MARIO NEGRI»

FONDAZIONE PER RICERCHE ERETTA IN ENTE MORALE CON D. P. R. 361 DEL 5 APRILE 1961 - REG. PERSONE GIUR. TRIB. MILANO N. 162, VOL. 5
CONTO CORRENTE POST. N. 38337205
COD. FISC. E PARTITA IVA 03254210150
ANAGRAFE NAZIONALE RICERCHE COD. G1690099

RECOGNIZED AS A TAX EXEMPT ORGANIZATION UNDER SECTION 501 (c) (3) OF THE UNITED STATES OF AMERICA INTERNAL REVENUE CODE
TAX I.D.No.: 98-6000957

AFOSR-TR- 87-1411

GRANT NUMBER : AFOSR - 85 - 0196

2.3.7.8-TETRACHLORODIBENZO-p-DIOXIN INDUCED IMMUNOSUPPRESSION : ITS POSSIBLE ALTERATION BY IN VIVO ADMINISTRATION OF SPECIFIC HEPATIC ENZYME INDUCERS

Annunciata Vecchi
"Mario Negri" Institute for Pharmacological Research
via Eritrea, 62 - MILANO, Italy

June 27, 1987

FINAL SCIENTIFIC REPORT : 1 May 1985 - 30 April 1987

Approved for public release; distribution unlimited

Prepared for

Air Force Office of Scientific Research, Bolling Air Force Base, DC, USA

and

European Office of Aerospace Research and Development - London - England

LIST OF ABBREVIATIONS AND SYMBOLS

TCDD	= 2.3.7.8-tetrachlorodibenzo-p-dioxin
TCDF	= 2.3.7.8-tetrachlorodibenzofuran
3MC	= 3-methylcholanthrene
βNF	= β-naphtoflavone
SRBC	= sheep red blood cells
PFC	= plaque forming cells
MLR	= mixed lymphocyte reaction
CTL	= cytotoxic T lymphocytes
IL-2	= interleukin 2
^{3}HTdR	= ^{3}H-methyl-thymidine
AHH	= aryl hydrocarbon hydroxylase
Et-De-Et	= ethoxy-resorufin de-ethylase

INTRODUCTION

The ultimate objective of this research program was to clarify whether drugs that in vitro inhibit TCDD binding to the hepatic cytosolic Ah "receptor" such as 3MC, βNF and TCDF could modify in vivo TCDD toxicity (evaluated as immunosuppression and enzymes induction). Because other TCDD toxic effects, thymic involution, porphyria and immunosuppression, have been shown to be related to the Ah locus which regulates AHH enzymes inducibility, examination of alterations of these enzymes has also been performed.

The study of this year has been concentrated on the effects of only two inducers, that is TCDF and 3MC, since phenobarbital has been shown to be ineffective on the parameters investigated and βNF was always less effective than 3MC , even if it has been shown (last report) to cause additive inhibition of the anti-SRBC response when given with TCDD. Studies presented in the annual report of last year have also shown that in combined treatments (TCDD and inducers, given at the same time) each drug did not influence the effect of the other. In fact, if drugs were active when given alone, their combination usually results in an additive effect, as seen for anti-SRBC antibody production and splenocyte blastogenesis in the presence of mitogens (see the report for details) . The indication that in combined treatments each drug did not influence the effect of the other has however been obtained with only one condition of treatment , that is simultaneous treatment, and only at one time of interval between treatment and tests. However, since the recovery of the effects of TCDD

and inducers on immunological as well as enzymatic parameters are quite different, a better understanding of the possible interaction between TCDD and inducers can be obtained with a time course study and modifing the sequence of drug administration.

On these basis, the following experimental conditions have been examined : 3MC has been given 7, 4 and 2 days before and 2 days after TCDD, in order to evaluate times when the enzymatic activities modified by 3MC are induced at different levels. Moreover TCDF was given 2 days before or after TCDD. Immunological and enzymatic tests were performed at different intervals as specified in the results.

Data on the effects of simultaneous treatment with TCDD and 3MC on MLR are also presented here, together with a preliminary analysis of the effect of TCDD on IL-2 production and CTL generation, in order to understand the basis of the additive inhibitory effect observed on MLR after TCDD-3MC treatment. Results obtained after simultaneous combined treatment with TCDD and TCDF as regards enzymatic induction are also included in this report.

RESULTS

In this study C57Bl/6CrBR male mice from Charles River, Calco, Italy, were used at 8 - 10 weeks of age. Each experimental group consisted of 7 and 6 animals respectively for immunological and enzymatic studies. Statistical analysis was always performed with the Duncan's test, unless otherwise specified in text or tables.

Drugs were given i.p. in the appropriate combination and sequence at different times before antigenic stimulation with SRBC; animals were always sacrificed 5 days after antigen injection. In order to have the best comparison for immunological and enzymatic results and to reduce the use of compounds as toxic as TCDD and TCDF, enzymatic parameters were usually evaluated in the liver of the same animals used for immunological studies. 3MC was dissolved in corn oil; TCDD and TCDF in acetone : corn oil mixture (1:6) to a concentration of 2 and 200µg/ml respectively and further diluted in corn oil to the desired working concentration.

Evaluation of immunological effects

a) humoral antiboby production

Antibody production was evaluated using the Cunningham's modification of the classical Jerne's technique (Vecchi et al., Chem. Biol. Interact., 30, 337, 1980).

Table 1 reports the results obtained with 4 different schedules of

TCDD-3MC treatments. The first experiment reported shows the results obtained when 3MC was given on day -7 and TCDD on day -5, that is 3MC precedes TCDD administration. In these conditions 3MC inhibited antibody production by about 80%, TCDD by about 60% and drug combination caused a suppression of more than 95%: the immunosuppression caused by combined treatment was thus the sum of the effects of each compound. Similar results were obtained when 3MC followed by 2 days TCDD administration on day -7 (second experiment reported in table 1). In fact in this condition 3MC reduced antibody production to 28% of control, TCDD to 53% and combination treatment to 6%. Since at these drug dosages (3MC 50mg/kg and TCDD 1µg/kg given either on day -7 or day -5) there was no difference in the cellularity of the spleen, the same level of reduction of the response was obtained if the results are expressed as PFC/spleen. If these results are considered together with those obtained with simultaneous administration of 3MC and TCDD on day -7, that were the sum of the effect of each drug (data presented in the previous report), it can be concluded that the sequence of drug injection is irrelevant for the final effect to be observed, at least when the interval between drugs is short. Next experiments were performed with a longer interval between 3MC and TCDD. Third experiment reported in table 1 shows the results obtained when 3MC was given 7 or 5 days before TCDD. It can be seen that 3MC induced immunosuppression recovered with time : in fact 3MC treatment on day -14 before antigenic stimulation inhibited antibody production of about 30%; this decrease was not always statistically

significant relative to control. In the experiment here reported only the value of PFC/spleen was significantly different from oil injected controls. A significant inhibition of antibody production was observed when 3MC was given on day -11: the level of inhibition was slightly higher than that observed on day -14, but the effect was consistent. When TCDD was given on day -7 to mice given 3MC on day -14 additive inhibition of antibody production was obtained. Same results were obtained with an interval of 5 days between 3MC and TCDD.

Next experiments were performed increasing the interval between drug combination treatment and time of antigen injection (table 2). 3MC was given 2 days before or after TCDD and SRBC were injected 21 days after treatment with the first given drug. Suppression of the response after TCDD treatment was still highly significant at day -21 as well as at day -19 day. On the other hand 3MC induced immunosuppression completely recovered at both times investigated. (-21 and -19). As regards combined treatment in both experimental conditions (3MC on day -21 and TCDD on day -19; TCDD on day -21 and 3MC on day -19) the inhibition of antibody production was the same as that of TCDD given alone at the time considered.

The effects of TCDF-TCDD combination are shown in table 3. Since in previous experiments (see report of last year) it was shown that doses of TCDF not active on immunological parameters (1 and 10μg/kg) did not modify TCDD induced immunosuppression when given in combination, in this set of experiments only the highest active dose of 100μg/kg TCDF was used. In the first experiment reported in table 3 the results obtained

with TCDF on day -5 and TCDD on day -7 are presented. It can be seen that TCDD and TCDF, given alone, were strong suppressants in these experimental conditions. Administration of both drugs increased the level of immunosuppression, inhibition of antibody production being about 60% for TCDD and TCDF and more than 90% with toxin combination. Similar results were obtained with TCDD on day -5 and TCDF on day -7 as shown in the second experiment reported in table 3. In these experimental conditions combined treatment, even if always results in PFC numbers lower than those of TCDD and TCDF given alone, did not always significantly reduce the response compared to the most active toxin given alone. The same situation has been observed also for TCDD - TCDF treatment given together on day -7 and it was considered in the preceding report.

A summary of the effects of 3MC - TCDD combination and TCDF - TCDD combination are shown in fig. 1. In the left panel of the figure the recovery after TCDF - TCDD combination given simultaneously is shown. Details of these experiments are in the report of last year. It can be seen that at both times investigated for combination treatments (-7 and -21) the inhibition caused by treatment with both toxins is higher than that induced by each drug. Moreover, the recovery from immunosuppression in animals treated with TCDF and TCDD parallels the recovery observed in mice treated with single drugs.

The right part of fig.1 shows the recovery after 3MC - TCDD combination, either when 3MC preceded or followed TCDD by 2 days. In both

experimental conditions it is clear that when 3MC induced immunodepression was recovered, the only detectable effect was that caused by TCDD.

Thus, on antibody production, it can be concluded that 3MC did not alter TCDD immunosuppression and that, when both drugs are immunosuppressive alone, their combination causes an additive inhibition of the immune response.

As regards TCDF - TCDD combinations results suggest an additive effect. However, in the various experiments performed with different schedules of treatments, the statistical analysis only occasionally shows significant differences between TCDF - TCDD treatment and the most depressive toxin investigated. There is the possibility that, because TCDF and TCDD have a very similar mechanism of action, if not the same, no additional effects can be obtained when significant inhibition is already present. However, in our experimental conditions, inhibition of humoral response was not complete, but in the order of 60% either with TCDD or with TCDF, thus a further decrease in the response was still possible.The reasons for this incomplete additive effects should be further investigated.

b) cell - mediated immune reactivities

Combination treatment with simultaneous 3MC-TCDD administration was tested also on cell-mediated immune reactivities. Results reported in the previous report showed that additional inhibition of the blastogenesis to Concanavalin A mitogen was observed when both compounds were given 12

days before testing. At the dosage used of 1µg/kg, TCDD was not active in modifying the blastogenic response to PHA and LPS and only 3MC at 50mg/kg significantly inhibited the response to both mitogens. In combination treatment the level of inhibition of PHA and LPS was the same as that of 3MC alone.

Here results obtained after combined treatment on MLR response are presented (table 4). This parameter evaluates the ability of lymphocytes to respond to alloantigens, instead of measuring polyclonal activation as was the case for mitogens; thus it can give further information on the ability of combined treatment to interfere with in vivo relevant immune reactivities. Experimental conditions were essentially as described (Bradley et al. in "Selected Methods in Cellular Immunology",p.162; B.B.Mishell and S.M.Shiigi eds., Freeman and Company, San Francisco 1980); briefly, $5x10^5$ splenocytes from C57Bl/6 mice ($H\text{-}2^b$) were mixed with $5x10^5$ X-rayed splenocytes from DBA/2 mice ($H\text{-}2^d$) in a volume of 0.2ml in RPMI 1640 medium plus 10% fetal bovine serum and incubated for 96 hours at 37°C in an atmosphere of 5% CO_2 in air. One µCi of ^{3}HTdR was added during the last 18 hours of incubation.

As shown in table 4, 3MC and TCDD significantly inhibited MLR response when given alone. In this set of experiments drugs were given 12 days before testing. These conditions are analogous to those used for testing the other parameters, humoral antibody production and enzyme induction, but mice were not challenged with SRBC. Animals treated with both drugs showed a further inhibition of MLR response: the final effect was actually

the sum of the effect of each drug, 3MC inhibiting the response by about 25%, TCDD by 15% and both drugs by 53%. In order to further investigate the phenomenon observed, an analysis of the possible mechanisms modified by drug treatment was performed. Data presented are at the moment limited to TCDD. In addition to MLR, the generation of cytotoxic T lymphocytes and IL-2 production after allogeneic stimulation were evaluated. CTL were induced incubating for 6 days at 1:1 ratio splenocytes from C57Bl/6 mice with X-rayed splenocytes from DBA/2 mice, then their ability to specifically kill allogeneic cells was evaluated incubating for 4 hours cultured C57Bl/6 splenocytes with ^{51}Cr labeled L1210 ($H\text{-}2^{d}$) leukemia cells and measuring the ^{51}Cr release in supernatants (Grabstein, in "Selected Methods in Cellular Immunology", p.126; B.B.Mishell and S.M.Shiigi eds, Freeman and Company, San Francisco, 1980). IL-2 released by splenocytes from C57Bl/6 mice incubated for 2 or 3 days with DBA/2 X-rayed splenocytes under the same conditions used for CTL induction was measured on the IL-2 dependent cell line CTLL 2. (Warren et al., Immunology 61, 167,1987). Table 5 shows that while MLR was inhibited by about 20%, a more marked inhibition, about 50%, was observed on CTL generation. At variance with inhibition induced by TCDD on MLR and CTL, the amount of IL-2 detectable in supernatants was significantly increased at both times investigated. Thus the impairment of cellular immune responses evaluated by MLR and CTL does not seem to be the consequence of a reduction in IL-2 availability. Even if other data are to be obtained before reaching a stable conclusion, it is reasonable to hypothesize that TCDD does not block IL-2 production, but rather it can render lymphocytes

unresponsive to it, interfering with IL-2 receptor on cell membrane, either by inducing the expression of a defective receptor or by preventing its maximal expression.

Studies on enzyme induction

The effect of combined treatments on hepatic microsomal cytochromes b_5 and P-448 content and on induction were evaluated using the same treatment schedules as for the determination of immunological parameters, in order to permit a correlation between results obtained in the two areas of investigation.

In a first group of experiments mice were treated with a scalar doses of TCDF (1 - 10 and 100 µg/kg) and TCDD; results are shown in table 6. Each compound was administered alone or in combination with TCDD, 7 days before antigen administration and mice were sacrificed 5 days after antigenic challenge. Cytochrome P-448 content was used as an index of microsomal enzyme induction still present at the time of killing after combined treatments. In addition it was also determined cytochrome b_5 content since it plays a role in the microsomal system of monooxygenases cytochrome P-448 dependent during monooxygenation. Using purified protein constituents in reconstituted metabolizing systems, evidence has

been obtained pointing to an interaction between cytochromes b_5 and P-450/P-448 which varies between being stimulatory or inhibitory depending on the P-450 form used, the choice of substrate and assay condition. In particular an obligatory b_5 requirement has been demonstrated for prostaglandin A, E_1 and E_2 hydroxylation by isozyme 2 of rabbit $P\text{-}450_{LM}$ via reconstituted system with phospholipid present.

Another well established function of this cytochrome is to behave as an electron carrier between NADH-cytochrome b_5 reductase and the terminal desaturase in the fatty acyl CoA desaturase system.

Microsomes were prepared according to Kato and Takayanaghi (Jnp. J. Pharmacol. 16, 380, 1966) and store at -80°C until enzyme assays. Cytochrome b_5 and P-448 were measured according to Omura and Sato (J. Biol. Chem. 239, 2370, 1964).

TCDF administration alone induced both cytochrome b_5 and P-448 in a dose related manner, with the lowest dose (1 µg/kg) showing no more inductive effects at the time of the sacrifice of the animals, the median dose (10µg/kg) causing a 42 and a 22% percentage increase respectively for cytochrome b_5 and P-448 and the highest dose (100µg/kg) causing significative induction of both cytochromes (130% for cytochrome b_5 and 83% for cytochrome P-448). TCDD administration alone caused significative induction of both cytochromes, of the same order of magnitude of that observed after treatment with 100 µg/kg TCDF.

Combined administration of the two compounds showed an additive effect on both cytochromes. In fact with the lowest dose of TCDF induction in the combined treatment group was comparable to that obtained after TCDD administration alone, while with the median and the highest dose of TCDF induction was slightly higher in the combined treatment group than that observed after TCDD administration alone ($p<0.05$); in this combined treatment level of induction of cytochrome P-448 was not different from that observed in the group treated with TCDF alone.

In another group of experiments it was investigated whether combined administration of TCDD and TCDF could alter hepatic induction observed after administration of each single compound, if the two compounds were administered at different time intervals before antigen administration. In one set of experiments mice were treated with TCDF (100µg/kg) or with TCDD (1µg/kg). The two compounds were given respectively 7 days (TCDF) or 5 days (TCDD) before antigen administration both alone and in combination. Animals were sacrificed 5 days after antigen administration. Besides cytochromes b_5 and P-448, it was determined also the activity of microsomal monooxygenases aryl hydrocarbon hydroxylase (AHH) and ethoxyresorufin-de-ethylase (Et-De-Et). AHH and Et-De-Et were measured respectively according to Nebert and Gelboin (J.Biol.Chem., 243, 6842, 1968) and to Burke and Mayer (Chem.Biol.Interact., 45, 243, 1983). Results are reported in table 7 and 8. TCDD and TCDF given alone caused comparable induction of cytochrome b_5, AHH and Et-De-Et, while in this experiment TCDD induced cytochrome P-448 more markedly than TCDF. In

combined treatment induction of all parameters in comparison to controls was lower than that observed with TCDD alone; for cytochrome b_5, AHH and Et-De-Et this effect was significative and indicated an interaction (inhibition) between the two compounds when the data were analyzed by two way analysis of variance and F interaction test. Significance of this finding would require further investigation, since doses of TCDD and TCDF used in this study were sufficiently high to be close to saturate the induction process, it could be impossible to appreciate further increases of induction.

In table 7 and 8 are also reported results obtained when TCDD and TCDF were given respectively 7 and 5 days before antigen administration. Induction observed when the two compounds were given alone was comparable to that observed in the experiment described previously with no significant difference in any parameter between the animals treated with TCDD or TCDF. In the combined treatment still for AHH activity and cytochromes b_5 and P-448 it was observed a significative interaction (inhibition); thus for this effect the two treatment schedules used (giving alternatively as first compound TCDD or TCDF) did not differ in an appreciable manner.

In table 9 and 10 are reported the results of combined treatment with 3MC and TCDD. Each compound was given alone or in combination 11 days (3MC 50mg/kg) or 7 days (TCDD 1µg/kg) before antigen administration. No change in comparison to control values was observed after treatment with

3MC in cytochrome b_5 and P-448 and in the activity of AHH and Et-De-Et, probably due to the long interval of time between inducer administration and sacrifice of the animals. Pretreatment with 3MC did not affect extent of induction of cytochrome b_5 and P-448 and of AHH and Et-De-Et activity observed after TCDD treatment, thus in the combined treatment we could not observe any synergism or inhibition between the two compounds on the parameters measured.

This suggest that in this case process of induction elicited by one compound didn't interfere with that caused by the other.

CONCLUSION

This research program has investigated the possibility that drugs able to interfere in vitro with the binding of TCDD to its specific cytosolic receptor could also modify TCDD toxicity in vivo, evaluated as immunosuppression and hepatic enzyme induction. Drugs with different ability to interfere with in vitro TCDD binding have been chosen. The order of potency as inhibitor is as follows: TCDF > 3MC > βNF > PB (phenobarbital). It must be added that PB was actually devoid of any effect on TCDD in vitro binding. When given in vivo, these compounds differ also for their inducing capacity of hepatic enzymes. In this report the term "cytochrome P-450" and "cytochrome P-448" are used to designate all form of cytochromes induced respectively by PB and polycyclic aromatic compounds; these are simplified expressions and in each case induction involves several isozymes. (Nebert et al. Biochem. Pharmacol. 31, 2311, 1982). With this statement in mind, in this study PB was used as a cytochrome P-450 inducer, 3MC, TCDF and TCDD as cytochrome P-448 inducers, while βNF was used as a mixed inducer, being able to increase the levels of either P-450 and P-448.

Here all the results obtained in the two years of the project are considered for final conclusions. In in vivo studies, at the dosages used, all compounds, but PB, are immunosuppressive on the parameter investigated. When combination experiments were performed, results obtained can be summarized as follows:

a) PB - TCDD combination induces the same immunosuppressive effect on

antibody production as TCDD given alone ;

b) Simultaneous administration of βNF and TCDD causes an inhibition of humoral response that is the sum of the immunosuppressive effect of each compound ;

c) 3MC and TCDD always caused additive immunodepressive effects on humoral antibody production, mitogen responsiveness and MLR;

d) Inhibition caused by TCDF - TCDD treatment usually is higher than that induced by each toxin alone. However the effect observed in a high proportion of experiments (about 70%) is not statistically different from that induced by the most active toxin in the conditions investigated.

As regards enzymatic induction, results are in the same direction as those obtained in immunological studies, and more specifically:

a) PB and βNF administration with TCDD slightly decreases the content of cytochrome P-448 relative to TCDD alone, but it does not modify the content of cytochrome b_5 and the induction of AHH;

b) 3MC given simultaneously or before TCDD does not change the hepatic content of cytochrome b_5 and cytochrome P-448 and the induction of AHH and Et-De-Et;

c) TCDF, given at the same time as TCDD and at a dose (100µg/kg) able to modify enzyme induction, significantly increases only the level of cytochrome P-448 over that caused by TCDD;

d) If TCDD is given at a 2 days interval relative to TCDD (either before or after) an interaction (inhibition) is observed.

Data obtained in this year in immunological studies substantiate our initial hypothesis that drugs able to interfere in vitro with TCDD and to induce the same enzymatic activities as TCDD do not appreciably interfere with TCDD induced immunological impairment. All the results obtained with 3MC given in different schedules and the time-course of the effect of combination treatment are consistent with this hypothesis. Evaluation of enzymatic induction is also in favor of independent effects for both compounds.

Interpretation of the results obtained with TCDF - TCDD combination is slightly more difficult, because immunodepressive and enzymatic effects are very marked and long lasting. In any case, the effects observed in immunological tests are in agreement with those on enzymatic induction. In fact, treatments which result in a negative interaction on enzymatic parameters are the same that do not cause additive inhibition on antibody production.

Studies here presented shows that TCDD toxicity, evaluated as immunosuppression and enzymatic induction, is not significantly decreased by administration of compounds able to induce the same enzymatic activities induced by TCDD. Rather, if the compounds are active alone the resulting toxic effect is usually the sum of the effect of each toxin. Moreover these data underlay once more that in vitro data, even suggestive and evaluated at a very fine level as is the specific binding of TCDD to its putative cytosolic receptor, need confirmation in in vivo systems where a lot of variables are at play.

Table 1

Effect of the sequence of 3MC and TCDD treatment on humoral antibody production

3MC 50mg/kg	TCDD 1 µg/kg	PFC/10^6 (%control)	PFC/spleen (%control)
–	–	863± 69 (100)	187013±19630 (100)
-7	–	158± 20 (18)**	17949± 3212 (6)**
–	-5	333± 26 (39)**	48760± 5681 (26)**
-7	-5	24± 4 (3)**◇◇	2362± 397 (2)**◇◇
–	–	742±109 (100)	79249± 8459 (100)
-5	–	206± 26 (28)**	17422± 3211 (22)
–	-7	393± 35 (53)*	43189± 6647 (54)*
-5	-7	46± 7 (6)**◇◇	3572± 638 (5)**◇◇
–	–	891± 80 (100	208010±20818 (100)
-14	–	633±118 (71)	139893±20350 (67)*
-11	–	533±133 (60)**	104495±27564 (50)**
–	-7	356± 32 (40)**	60984± 7178 (29)**
-14	-7	252± 37 (28)**	37162± 5608 (18)**
-11	-7	246± 17 (28)*	36543± 5588 (18)*

*p<0.05 relative to control

** p<0.01 relative to control

* p<0.05 relative to 3MC

** p<0.01 relative to 3MC

◇◇ p<0.01 relative to TCDD

Table 2

Effect of the sequence of 3MC and TCDD treatment on humoral antibody production

3MC 50mg/kg	TCDD 1μg/kg	PFC/10^6(%control)	PFC/spleen(%control)
–	–	664±39 (100)	134958± 9241 (100)
-21	–	760±25 (114)	167852±11481 (124)*
–	-19	332±30 (50)**	59636± 5404 (44)**
-21	-19	472±29 (62)*°	88639± 4527 (66)*°
–	–	960±87 (100)	199943±23463 (100)
-19	–	863±67 (90)	150008±12282 (75)
–	-21	611±39 (64)**	98308±15785 (49)**
-19	-21	560±38 (58)*°	88861± 9030 (44)*°

*p<0.05 relative to control

** p<0.01 relative to control

° p<0.05 relative to 3MC

°° p<0.01relative to 3MC

Table 3

Effect of the sequence of TCDD and TCDF treatment on humoral antibody production

TCDF 100 µg/kg	TCDD 1 µg/kg	PFC/10^6(%control)	PFC/spleen(%control)
–	–	520±176 (100)	92884±34836 (100)
-5	–	195± 75 (38)**	34042±12621 (37)**
–	-7	200± 79 (38)**	36991±15541 (40)**
-5	-7	35± 11 (7)**	5271± 1498 (6)**●
–	–	773± 77 (100)	128144± 9513 (100)
-7	–	308± 71 (40)**	61596±14774 (48)**
–	-5	231± 30 (30)**	38574± 5139 (30)**
-7	-5	97± 44 (14)**●	15076± 6985 (14)**●●

**p<0.01 relative to control

●p<0.05 relative to TCDF

●●p<0.01 relative to TCDF

Table 4

Effect of combined treatments of 3MC and TCDD on mixed lymphocyte reaction

treatment	c p m (R --> S*)	c p m (R --> R*)
oil	36609 ± 787	1657 ± 102
3MC 50 mg/kg	28465 ± 856*	2267 ± 354
TCDD 1 µg/kg	32139 ± 1056*	1349 ± 52
3MC + TCDD	19813 ± 1106°◇	769 ± 66*

* $p < 0.01$ relative to control (oil)

° $p < 0.01$ relative to 3MC

◇ $p < 0.01$ relative to TCDD

Table 5

Effect of TCDD on cell - mediated responses

	MLR +		CTL*		IL-2 production#	
treatment	R-->S◇	R-->R◇	25:1	50:1	+2	+3
oil	13108 ±801	1686 ± 172	34.8 ± 1.6	50.7 ±1.0	14350 ± 972	4249 ±390
TCDD 1 µg/kg	10494* ± 845	1308 ± 142	17.1** ± 0.3	27.4** ± 0.5	24105** ± 531	7561** ±851

+ Results of MLR are reported as counts per minute (^{3}H-thymidine) incorporated by C5[illegible] splenocytes (R) stimulated with DBA/2 X-rayed (S◇) splenocytes

* Results of CTL are reported as the specific percentage of ^{51}Cr released from L1210 labeled target cells in a 4 hour assay

IL-2 released on day 2 and 3 by C57Bl/6 splenocytes stimulated as in MLR was tested on CTLL 2 cells, an IL-2 dependent cell line. Results reported are counts per minute of ^{3}H-thymidine incorporated by 10^{4} CTLL 2 cells; dilution of the supernatants was 1:2.

*p<0.05 by Student's "t" test

**p<0.01

Table 6

Effect of combined treatment with TCDF and TCDD on day-7 on cytochrome b_5 and P-448 content

TCDF µg/Kg	TCDD µg/Kg	Cyt - b_5 (nmol/mg protein)	Cyt P-448 (nmol/mg protein)
-	-	0.233±0.012	0.436±0.020
-	1	0.330±0.026	0.638±0.056*
1	-	0.230±0.019	0.529±0.062
1	1	0.292±0.014	0.654 ±0.062*
-	-	0.285±0.015	0.472±0.031
-	1	0.405±0.024**	0.855±0.092**
10	-	0.364±0.020*	0.579±0.030
10	1	0.479±0.018*	1.029±0.058**
-	-	0.262±0.024	0.560±0.021
-	1	0.570±0.031**	0.909±0.078*
100	-	0.605±0.029**	1.030±0.093**
100	1	0.658±0.030**	1.227±0.106**•

*p<0.05 relative to control

** p<0.01 relative to control

• p<0.05 relative to TCDD

Table 7

Effect of combined treatment with TCDF and TCDD on cytochrome b_5 and P-448 content

TCDF 100µg/kg	TCDD 1µg/kg	Cyt - b_5 (nmol/mg protein)	Cyt P-448 (nmol/mg protein)
-	-	0.335±0.030	0.641±0.047
-7	-	0.507±0.011**	0.772±0.070
-	-5	0.547±0.037**	1.018±0.043**@
-7	-5	0.474±0.053*	0.907±0.097*
-5	-	0.526±0.073	0.948±0,065**
-	-7	0.546±0.019*	1.051±0.038**
-5	-7	0.460±0.086	0.821±0.059**••

*p< 0.05 relative to control

** p< 0.01 relative to control

@p<0.05 relative to TCDF -7

•• p<0.01 relative to TCDD -7

Table 8

Effect of combined treatment with TCDF and TCDD on hepatic induction

TCDF 100µg/kg	TCDD 1µg/kg	AHH (pmol/min/mg prot.)	Et - De - Et (µmol/min/mg prot.)
-	-	73.742± 1.90	0.054±0.007
-7	-	1491.00±215.12**	4.745±0.646**
-	-5	1451.72±182.73**	5.879±1.362**
-7	-5	1404.86±109.14**	6.063±1.162**
-5	-	1290.07±171.84**	4.200±1.001*
-	-7	1576.90± 53.85**	4.857±0.441**
-5	-7	1751.30±392.40**	7.247±1.362**

*p<0.05 relative to control

** p<0.01 relative to control

Table 9

Effect of combined treatment with 3MC and TCDD on cytochrome b_5 and P-448 content

3MC 50mg/kg	TCDD 1 µg/kg	Cyt - b_5 (nmol/mg protein)	Cyt P-448 (nmol/mg protein)
-	-	0.259±0.007	0.652±0.067
-11	-	0.356±0.034	0.634±0.055
-	-7	0.554±0.025** °°	1.108±0.061** °°
-11	-7	0.569±0.054** °°	1.136±0.094** °°

** $p < 0.01$ relative to control

°° $p < 0.01$ relative to 3MC

Table 10

Effect of combined treatment with 3MC and TCDD on hepatic induction

3MC	TCDD	AHH	Et - De - Et
50mg/kg	1 µg/kg	(pmol/min /mg prot.)	(µmol/min/mg prot.)
-	-	72.17± 8.26	0.036±0.005
-11	-	157.81± 12.99	0.053±0.006
-	-7	1677.83±131.55****	4.512±0.471****
-11	-7	1576.91±142.62****	5.261±0.341****

**p <0.01 relative to control

** p <0.01 relative to 3MC

Conference

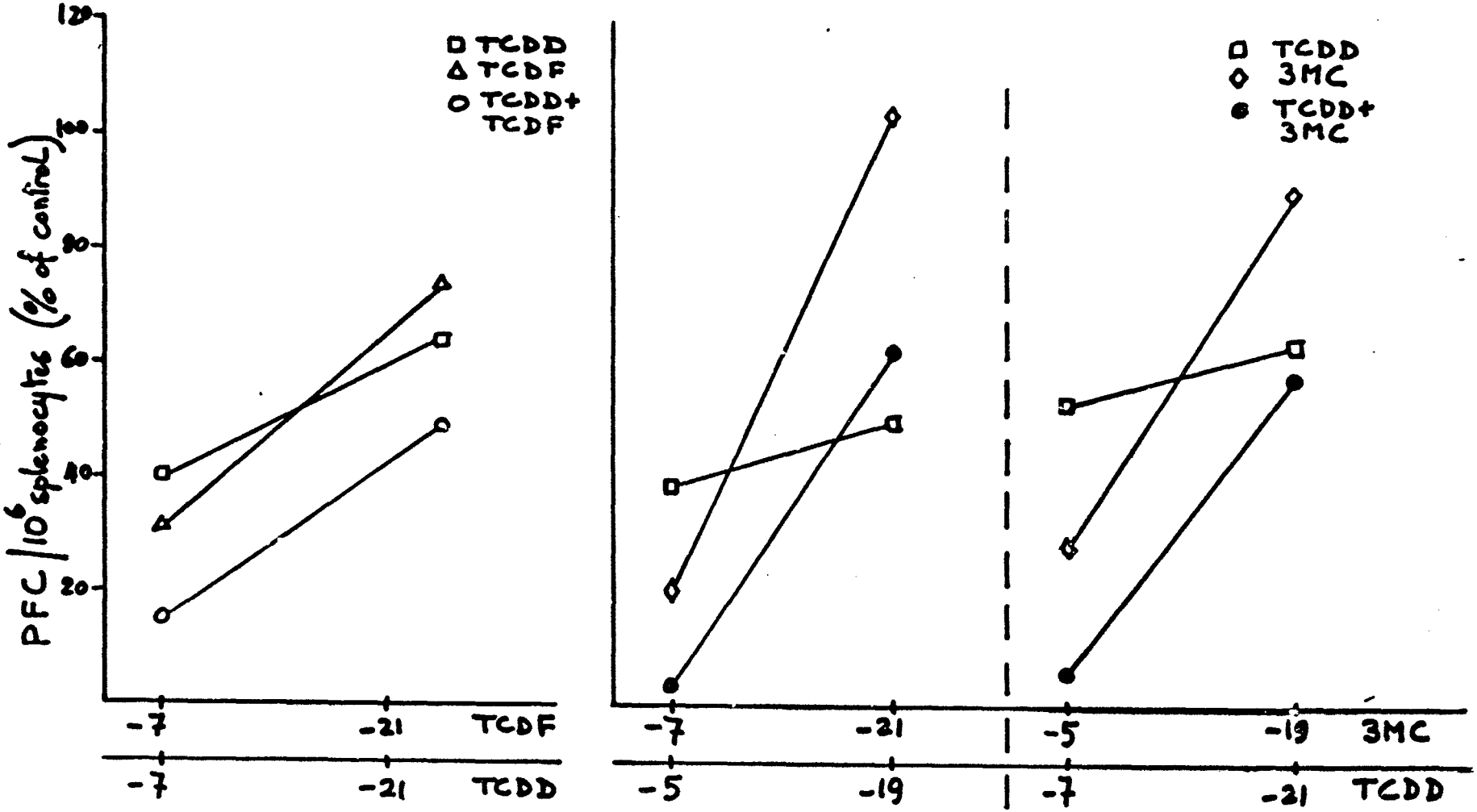
The principal investigator attended the "Dioxin 85 - 5th Int. Symp. on Chlorinated Dioxins and related Compounds", Sept. 16-19, Bayreuth (FRG) 1985.

She received a travel support for attending the meeting from the Italian Ministry of Foreign Affairs (Ministero degli Affari Esteri) Rome, Italy.

LEGEND

FIG. 1 : Time - course of the effect of different schedules of TCDD-inducers combination on humoral antibody production

FIG 1


PFC / 10^6 splenocytes (% of control)
120
100
80
60
40
20
TCDD
TCDF
TCDD+ TCDF
-7
-21
TCDF
-7
-21
TCDD
TCDD
3MC
TCDD+ 3MC
-7
-21
-5
-19
3MC
-5
-19
-7
-21
TCDD

Journal articles

Part of the work summarized in this report has been published in Chemosphere, Vol. 15, pg. 1707-1714, 1956
(enclosed)